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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Dose escalation using carbamylated monomeric tree pollen drops is well tolerated in patients with allergic rhinoconjunctivitis and points towards clinical effects

To the Editor,

Pollen allergy is currently affecting about 40% of all allergic patients in Europe and the prevalence is increasing, with birch (*Betula*) being the most common tree pollen allergen.¹ In most cases, pollen allergy induces intermittent rhinoconjunctivitis, which can worsen into a persistent form and allergic asthma, if not treated properly.² Guidelines recommend allergen immunotherapy (AIT) for patients with allergic rhinoconjunctivitis regardless of concomitant asthma. The sublingual route of AIT, SLIT, showed to be generally effective, safe and well tolerated in patients with allergic rhinitis, conjunctivitis or both.³ Safety of SLIT can be improved with allergoids: in contrast to native allergens, polymerized allergoids have a lower allergenic potential, which reduces the risk of allergic reactions.⁴ Differently, carbamylated monomeric allergoids, which have selective amino acid substitution to limit IgE binding, are 25-80 times smaller than allergoids, making them suitable for SLIT. They are less susceptible to enzymatic degradation,^{5,6} enabling the remaining swallowed amount of extract to reach the gut-associated lymphoid tissue, exerting additional immune stimulation.^{4,5}

Here, we present the results of a phase IIa randomized, double-blind, placebo-controlled dose escalation trial to demonstrate the safety and feasibility of a dose of 50 000 UA per day of SLIT with tree pollen drops consisting of 50:50 carbamylated monomeric birch and alder pollen allergoid.

The study ran from January to June 2018 in 5 centres in Germany. After having given written informed consent, 21 eligible patients (6 placebo and 15 actively treated patients) (Appendix S1: consort flow, Appendix S2: baseline characteristics) were randomized and treated for 70 days. Up-dosing was performed preseasonally within 8 days, starting with 2000 UA (units of allergen, Appendix S3) and increasing to 50 000 UA/day in 5 steps over 3 visits, with a short home interphase between the visits (Appendix S3). This was followed by a maintenance phase with 50 000 UA/day for 62 days. Safety and clinical tolerability of tree pollen sublingual drops were assessed through documentation of solicited (expected local reactions at the administration site according to Passalacqua et al⁷) adverse drug reactions (ADRs) and systemic allergic reactions.⁸ Clinical and immunological effects of the treatment were investigated through the proportion

Day	Visit	Dose of tree pollen drops [UA]	N (%) Solicited local reactions	N (%) Unsolicited local reactions	N (%) Systemic reactions
1	V2 ^a	2000	1 (0.11%)	1 (0.11%)	-
		5000	2 (0.22%)	2 (0.22%)	-
		10 000	1 (0.11%)	-	-
2-3	h ^b	10 000	-	-	-
4	V3 ^a	15 000	-	-	-
		25 000	-	-	-
5-7	h ^b	25 000	6 (0.65%)	-	6 (0.65%)
8	V4 ^a	50 000	-	-	-
9-71	m ^b	50 000	-	-	-

In total, 920 doses of tree pollen sublingual drops were administered.

Abbreviations: h, home interphase between up-dosing visits; m, maintenance phase after up-dosing; UA, units of allergen; V, visit.

^aAdministration at the doctor's office.

^bSelf administration at home.

TABLE 1 Adverse drug reactions after treatment with tree pollen sublingual drops

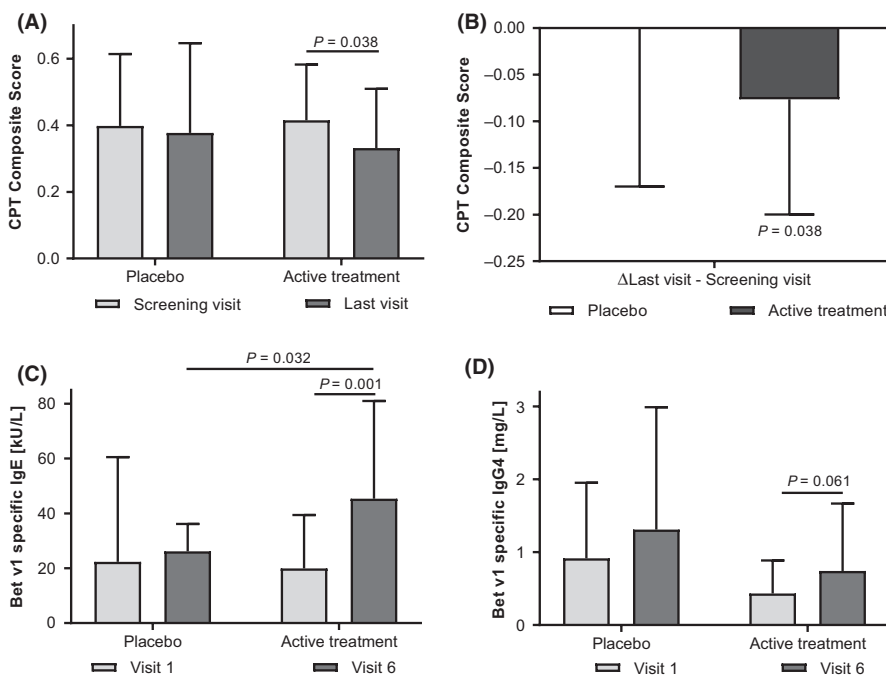


FIGURE 1 Clinical effects of tree pollen sublingual drops. (A) CPT composite score (Astvatsatourov et al⁸) before and after treatment, (B) Delta (Δ) last visit–screening visit of the CPT composite score, (C) sIgE levels and (D) sIgG4 levels at V1 and V6. Data are expressed as mean + SD. CPT, conjunctival provocation test; kU, kilo units; L, litre; mg, milligram; SD, standard deviation

of patients showing a reduction in CPT (conjunctival provocation test) composite score (CS,⁹ Appendix S5) after treatment compared with baseline and the changes in serum levels of birch pollen-specific immunoglobulins E (sIgE) and G4 (sIgG4).

Overall, no fatality nor serious ADR occurred during the trial. No epinephrine was used. The intended cumulative dose of 3 302 000 UA $\pm$ 30% was received by 13 patients (86.6%). Systemic allergic reactions were documented for one actively treated patient: after up-dosing to 25 000 UA at the doctor's office without any ADR, the patient experienced 6 grade I systemic reactions (rhinitis) along with 6 solicited local reactions (scratchy throat, Table 1) during the subsequent home interphase (Table 1). In consequence, the patient dropped out at the end of the escalation phase. Apart from that, no systemic reaction grade II or higher was recorded in the course of the

study. One patient experienced aggravated rhinoconjunctivitis symptoms because of increasing pollen load at the onset of the season and hence discontinued AIT at day 8.

Solicited and unsolicited (unexpected) local reactions occurred in 1.4% of the 920 total administrations and—except for the 6 local reactions experienced by one patient mentioned above—exclusively at the lowest three up-dosing steps and solely at the first treatment visit: four solicited and 3 unsolicited local reactions were documented for two patients 30 minutes after up-dosing at the doctor's office (Table 1, Appendix S4). No solicited or unsolicited ADR was observed in the subsequent maintenance phase with 50 000 UA/per day.

Reactivity of the conjunctival mucosa, as determined by the CS, was slightly reduced by 7.5% from the screening visit to the last

visit in the placebo group, while it significantly decreased by 19.5% ($P = 0.038$, Figure 1A) in the actively treated group.

Concerning immunogenicity parameters, sIgE increased moderately (1.2-fold) in the placebo group over the course of the study. In the actively treated group, sIgE levels were 2.3-fold higher ($P = 0.001$) after treatment than before treatment (Figure 1B). Serum sIgG4 increased by 43% ($P = 0.173$) in the placebo and by 71% ($P = 0.061$, Figure 1C) in the actively treated group.

In this first-in-patient study, we demonstrate that the administration of a carbamylated monomeric tree pollen allergoid escalated to a maximal daily dose of 50 000 UA is safe and well tolerated in patients with birch pollen-induced allergic rhinoconjunctivitis. Only one patient was withdrawn from the trial because of mild systemic reactions. The few local reactions that occurred during the up-dosing phase were all of mild severity and did not require dose adjustments. All patients with local reactions—except the one with concurrent systemic reactions—reached the maximal dose without any further ADRs and completed the trial as scheduled in the protocol. In particular, no solicited or unsolicited ADR occurred in the subsequent maintenance phase of 62 days with 50 000 UA/day. This confirms the well-known safety profile of the allergoid even at previously unthinkable high doses.

In addition to the safety aspects, this study provides interesting data on clinical and immunological effects of the treatment despite the small number of included patients. The analysis of response to CPT showed a significant reduction for the active but not for the control group, hinting at clinical improvement. The statistically significant increase for sIgE and the nearly statistically significant increase for sIgG4 in the actively treated, but not in the placebo group, are both in accordance with a recently published paper by Couroux et al, who showed similar results for birch pollen sIgE and sIgG4 after 10 weeks of treatment with a sublingual birch tablet.¹⁰ However, the preliminary findings presented here suggest that a longer treatment duration in a larger sample size is desirable to properly investigate the extent and relevance of the clinical and immunological changes induced by this treatment.

In conclusion, this early phase clinical dose escalation study has successfully demonstrated the safety and feasibility of this sublingual allergen immunotherapy with the carbamylated monomeric tree pollen allergoid up to a dose of 50 000 UA per day. The clinical effect of this high-dose concept has to be established in a larger dose-finding study.

CONFLICTS OF INTEREST

ER, JS and CA have nothing to disclose. RM reports personal fees from ALK, grants from ASIT biotech, personal fees from allergopharma, personal fees from Allergy Therapeutics, grants and personal fees from Bencard, grants from Leti, grants, personal fees and nonfinancial support from Lofarma, nonfinancial support from Roxall, grants and personal fees from Stallergenes, grants from Optima, personal fees from Friulchem, personal fees from Hexal, personal fees from Servier, personal fees from Klosterfrau, nonfinancial support from Atmos, personal fees from Bayer,

nonfinancial support from Bionorica, personal fees from FAES, personal fees from GSK, personal fees from MSD, personal fees from Johnson&Johnson, personal fees from Meda, personal fees and nonfinancial support from Novartis, nonfinancial support from Otonomy, personal fees from Stada, personal fees from UCB, nonfinancial support from Ferrero, grants from BitopAG, grants from Hulka, personal fees from Nuvo and grants from Ursapharm, outside the submitted work. SA reports grants and personal fees from Lofarma, personal fees from Servier, personal fees from Hexal and personal fees from Friulchem, outside the submitted work. EC is an employee of Lofarma SpA

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ETHICS APPROVAL

The trial upon which this analysis was based was approved by the Medical Ethics Committee of the North Rhine Medical Council (reference number 2017318) and the German Federal Ministry of Health (Paul-Ehrlich-Institut, Eudra-CT number 2017-003063-34). Patients in this study were required to give written informed consent before enrolment.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

N=37
Patients screened

N=15
Screening Failures:
slgE <0.7 kU/L (N=15)

N=21
Patients randomized

N=1
Withdrawal of consent

N=6
Placebo

N=15
Tree pollen drops

N=6 Completed	
N=2 PP	N=6 ITT

N=0
Drop out

N=13 Completed	
N=7 PP	N=13 ITT

N=2 Drop out	
N=0 PP	N=2 ITT

Additional File 2

Table: Baseline characteristics at screening visit

a) Duration of Rhinoconjunctivitis (years)

	Mean	N	SD	Median	Minimum	Maximum
Tree pollen drops	9.3182	15	5.74	8	4	22
Placebo	12.6019	6	12.78	8	4	38
Total	10.2564	21	8.14	8	4	38

b) ARIA results

		Placebo		Tree pollen drops	
		N	Percent	N	Percent
Are the rhinitis symptoms troublesome?	Yes	6	100	15	100
	No	0	0	0	0
Do the rhinitis symptoms cause impairment of the patient's daily activities, in sports, at leisure?	Yes	6	100	15	100
	No	0	0	0	0
Do the rhinitis symptoms cause impairment at work or at school?	Yes	6	100	13	86.7
	No	0	0	2	13.3
Do the rhinitis symptoms disturb the patient's sleep?	Yes	4	66.7	11	73.3
	No	2	33.3	4	26.7
Intermittent (symptoms are present < 4 days a week or for < 4 weeks in the year)		1	16.7	2	13.3
Persistent (symptoms are present > 4 days a week and for > 4 weeks in the year)		5	83.3	13	86.7

c) SPT results (mm)

			Negative Control	Histamine Control	Birch Pollen	House dust mites	Cat Epithelia	Dog Epithelia	Hazel
Placebo	N	Valid	6	6	6	6	6	6	6
		Missing	0	0	0	0	0	0	0
	Mean		0.00	4.00	5.83	1.33	1.83	1.50	3.83
	Median		0.00	4.00	5.50	1.50	2.00	1.50	3.00
	Std. Deviation		0.000	0.894	1.722	0.816	1.835	1.517	2.401
	Minimum		0	3	4	0	0	0	2
	Maximum		0	5	9	2	5	4	8
Tree pollen drops	N	Valid	15	15	15	15	15	15	15
		Missing	0	0	0	0	0	0	0
	Mean		0.00	4.53	7.47	1.33	1.40	1.07	2.0
	Median		0.00	4.00	5.00	2.00	2.00	2.00	2.00
	Std. Deviation		0.000	1.187	6.490	1.291	1.183	1.033	1.309
	Minimum		0	3	4	0	0	0	0
	Maximum		0	7	30	4	4	2	4

d) CPT reactivity score

			V1
Placebo	N	Valid	6
		Missing	0
	Mean		2.33
	Median		2.00
	Std. Deviation		0.516
	Minimum		2
	Maximum		3

Tree Pollen drops	N	Valid	15
		Missing	0
	Mean		2.47
	Median		2,00
	Std. Deviation		0.516
	Minimum		2
	Maximum		3

e) Immunologic parameters

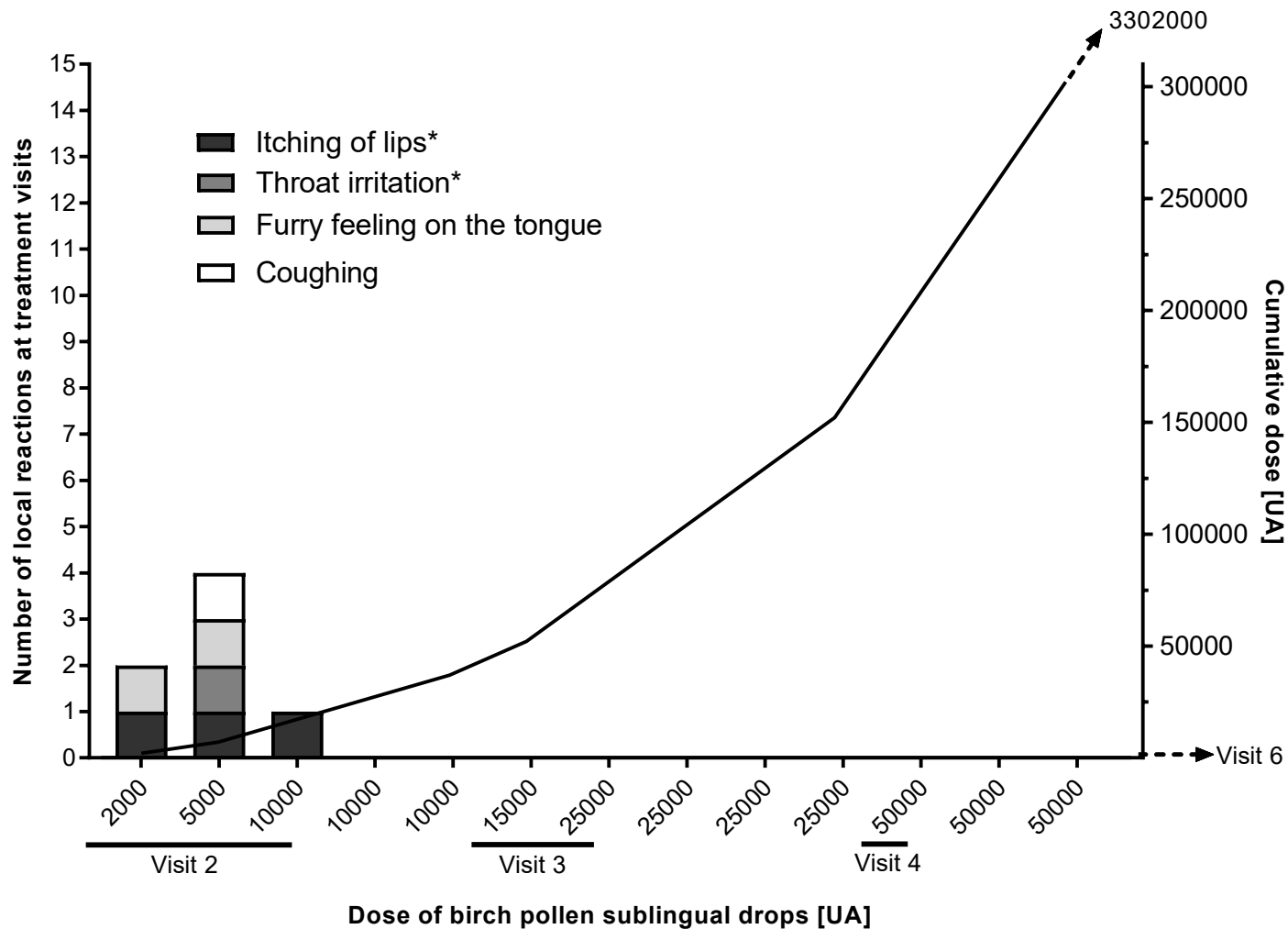
		Bet v1 specific		
		IgE [kU/L]	CAP class	IgG4 [mg/L]
Tree pollen drops	N	14	14	14
	Mean	19.971	3.50	0.434
	Median	12.000	3.00	0.230
	Std. Deviation	19.472	0.855	0.454
	Minimum	0.930	2	0.070
	Maximum	59.100	5	1.390
Placebo	N	6	6	6
	Mean	22.322	3.33	0.917
	Median	8.355	3.00	0.470
	Std. Deviation	38.222	1.366	1.036
	Minimum	1.690	2	0.070
	Maximum	100.000	6	2.440

Additional File 3: Tabular display of the treatment phase.

h = home interphase between updosing visits, m = maintenance phase after updosing; UA: Units of allergen (One Unit of Allergen corresponds to 1/40 of the mean provocation dosage of the comparable unmodified allergen determined by nasal provocation testing in allergic rhinitis patients.)

Day	Visit	Number of drops	Concentration of tree pollen drops [UA/mL]	Dose of placebo [UA]	Dose of tree pollen drops [UA]
1	V2 ^a	2	20,000	0	2,000
		5	20,000	0	5,000
		2	100,000	0	10,000
2 to 3	h ^b	2	100,000	0	10,000
4	V3 ^a	3	100,000	0	15,000
		5	100,000	0	25,000
5 to 7	h ^b	5	100,000	0	25,000
8	V4 ^a	10	100,000	0	50,000
9 to 71	m ^b	10	100,000	0	50,000

^a: administration at doctor's office, ^b: self-administration by the patient at home



Additional File 5: Principle of the CPT (Conjunctival Provocation Test) Composite Score (CS)

The investigator had to assess the severity score at each provocation step based on Gronemeyer's grading scale:

Severity Score (s_i)	Findings
0	No subjective or visible reaction
1	Itching, reddening, foreign body sensation
2	Stage I and in addition tearing, vasodilation of conjunctiva, bulbi (threshold value)
3	Stage II and in addition vasodilation and erythema of conjunctiva tarsi, blepharospasm
4	Stage III and in addition chemosis, lid swelling

Gronemeyer's grading scale to assess severity scores in CPT (Riechelmann et al. 2003)

If the severity score s_i had not reached the threshold value s_{th} ($s_i = s_{th} \geq 2$) and the CPT had been considered as negative, the provocation had to be repeated with an increased concentration in the corresponding visit.

The CPT analysis was performed using two different scores, the composite score and the reactivity score.

The composite score (CS) is a combination of all severity scores at a corresponding visit and was calculated for each patient at each visit using the following equation:

$$CS = \frac{\sqrt{\sum_{i=1}^n s_i^2}}{n^2}$$

n = Number of provocation steps; s_i = severity score

This score was used to analyse the improvement of the CS between V1 and V6 and to compare the improvement between the actively treated and placebo group.

The reactivity score was quantified using the following system:

4 = Reaction at 1:1000 dilution of provocation solution

3 = Reaction at 1:100 dilution of provocation solution

2 = Reaction at 1:10 dilution of provocation solution

1 = Reaction at undiluted provocation solution

0 = No reaction

This score was used to analyse the improvement of CPT-reactivity between V1 and V6 and to compare the improvement between actively treated and placebo group using the following scoring system:

-2 = Worsening of two allergen concentration steps

-1 = Worsening of one allergen concentration step

0 = No change

+1 = Improvement of one allergen concentration step

+2 = Improvement of two allergen concentration steps

+3 = Improvement of three allergen concentration steps

+4 = Improvement of four allergen concentration steps